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Serum lipids prior to starting androgen deprivation therapy and risk of castration-resistant prostate cancer and metastasis: Results from the SEARCH Database

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ABSTRACT

Purpose: To test the association between serum lipid levels prior to androgen deprivation therapy (ADT) and risk of CRPC and metastasis.

Materials and methods: We identified 302 men in the SEARCH database who received ADT after radical prostatectomy for non-metastatic disease, never used statins before ADT, and had available serum lipid data within two years prior to ADT. Cox proportional hazards models were used to test associations between total cholesterol (<200 vs. \geq 200 mg/dl), low-density lipoprotein (LDL; <130 vs. \geq 130 mg/dl), high-density lipoprotein (HDL; \geq 40 vs. <40 mg/dl) and triglycerides (<150 vs. \geq 150 mg/dl) and risk of CRPC and metastasis after ADT, adjusting for potential confounders. In subanalyses, we restricted to men who remained statin non-users post-ADT.

Results: Median follow-up was 67 months; 42 men developed CRPC and 44 metastasis. Men with elevated cholesterol had earlier ADT year and longer follow-up, and higher rates of statin use post-ADT. In multivariable analysis, total cholesterol and LDL were unrelated to CRPC. Low HDL (<40 vs. \geq 40mg/dL) was suggestively linked with increased risk of CRPC (HR 1.86; 95%CI 0.99-3.48), with a stronger association in men who remained statin non-users post-ADT (HR 3.64; 95%CI 1.45-9.17). Results for metastasis were similar to those found for CRPC.

Conclusions: Among men with non-metastatic prostate cancer starting ADT, serum cholesterol was unrelated to CRPC or metastasis. Low HDL was suggestively associated with increased CRPC and metastasis risk, particularly in statin never-users. Further studies are needed to explore a potential role for lipids in prostate cancer progression after ADT.

INTRODUCTION

Prostate cancer is the most common non-cutaneous cancer and the second leading cause of death in US men.¹ Despite widespread early responses to androgen deprivation therapy (ADT), the primary treatment for advanced and metastatic prostate cancer, a subset of tumors almost invariably progress to castration-resistant prostate cancer (CRPC). Although serum androgens are low in men with CRPC, tumor androgens in men on ADT can be sufficient to activate the AR, and a key source of intracellular androgen is *de novo* steroidogenesis from intratumoral cholesterol.²⁻⁴

A number of epidemiological studies found a link between higher serum cholesterol and increased risk of advanced prostate cancer and/or progression.^{3, 5, 6} Furthermore, mounting evidence supports an inverse association of the cholesterol-lowering statins with advanced and lethal prostate cancer risk.⁷ Among men starting ADT, one study found that statin users had delayed time to CRPC.⁸ Moreover, *in vitro* experiments showed that statins inhibited uptake of testosterone precursor dehydroepiandrosterone sulfate by competitively binding transporter SLCO2B1.⁸ A large observational study reported that statin use alongside ADT was associated with reduced prostate cancer-specific and all-cause mortality.⁹ Collectively, these data suggest the potential of targeting cholesterol homeostasis at the time of ADT to improve outcomes.

Despite strong biological rationale supporting a potential role for high cholesterol in CRPC, only one epidemiological study has examined this to date.¹⁰ The aim of our study was to examine whether lipid levels prior to ADT were associated with CRPC and metastasis risk after ADT. To test this, we used data from a retrospective cohort of prostate cancer patients in the Shared Equal Access Regional Cancer Hospital (SEARCH) database who received post-operative ADT without known metastatic disease. Our primary hypothesis was that elevated cholesterol would be associated with increased CRPC risk.

MATERIALS AND METHODS

Study sample

After obtaining Institutional Review Board approval, data from patients undergoing radical prostatectomy (RP; n=7,811) from 1982-2017 at eight Veterans Administration (VA) Medical Centers were combined into the SEARCH database.¹¹ SEARCH does not include patients treated with preoperative ADT or radiation therapy. Given our goal to understand if serum lipids at ADT were associated with CRPC or metastasis risk, we excluded patients who never received ADT (n=6,466). As illustrated in Supplementary Figure 1, we excluded men who started statins before ADT, for a more accurate measure of serum cholesterol (n=653). Of 692 non-statin users who started ADT, we excluded patients with missing serum lipids (n=320), missing PSA at ADT (n=36), patients with metastases by the time of ADT (n=27), missing race (n=3), and missing pathology results (n=4), resulting in 302 men.

Exposure assessment and definitions

Fasting serum lipids (total cholesterol, LDL, HDL and TG) measured within two years prior to ADT were abstracted from VA computerized medical records. The VA Informatics and Computing Infrastructure (VINCI) enabled more complete abstraction of lipid data from medical records after 2000. As such, men excluded due to missing lipids had less recent year of ADT (Supplementary Table 1). In addition, these men tended to be older at ADT, were less likely to be black, had higher PSA at surgery and ADT, and were more likely to have received radiation. For men with available lipid data, recommended cut-offs for normal vs. abnormal serum levels (all in mg/dl) were selected based on National Cholesterol Education Program (NCEP)-Adult Treatment Panel (ATP) III guidelines.¹²

Follow-up and outcome ascertainment

Follow-up protocols were at the discretion of treating physicians. Pre-ADT PSA doubling time (PSADT) was calculated according to Prostate Specific Antigen Working Group guidelines.¹³ CRPC (primary outcome) was defined using Prostate Cancer Working Group Two criteria.¹⁴ Imaging reports (bone scan, magnetic resonance imaging, computed tomography, x-ray) after surgery were assessed by trained personnel to determine the development of metastases (secondary outcome).

Statistical analysis

The characteristics of men excluded due to pre-ADT statin use are presented in Supplementary Table 2. Excluded men were slightly older than those included in our analysis, with higher BMI and longer time between surgery and start of ADT. In addition, excluded men had lower total cholesterol, LDL and HDL but higher triglycerides. Pathological characteristics did not differ between excluded and included men.

In line with our primary hypothesis, analysis of total cholesterol was considered primary, while analyses of LDL, HDL and TG were considered secondary. Demographic, clinical and pathologic differences between patients with normal vs. abnormal total cholesterol (<200 vs. ≥ 200 mg/dl) were examined using descriptive statistics.

Time from ADT initiation to CRPC was compared between normal vs. abnormal serum cholesterol groups using Kaplan-Meier plots and the log-rank test. Cox proportional hazards analysis was used to test whether serum cholesterol levels (abnormal vs. normal, and continuous in 10mg/dl increments) were associated with time to CRPC. Forward selection with entry threshold of $p < 0.05$ was used to prevent overfitting. Candidate covariates included PSA at surgery and at ADT (continuous, log-transformed), pathological grade group (1, 2-3, 4-5), positive surgical margins, extracapsular extension, seminal vesicle invasion, positive lymph nodes, age and year at ADT, race (black vs. non-black), statin use after ADT (time-dependent), radiation treatment after ADT (time-dependent), time from surgery to ADT, and PSADT (<9 months, ≥ 9 months, unknown). Selected covariates are listed in Table footnotes. In sensitivity analysis, given the potential role for obesity in CRPC,¹⁵ we forced BMI into our models and though this made estimates less precise, it did not appreciably change our results. In secondary analysis using the same approach as described for total cholesterol, we tested associations between LDL, HDL and TG and CRPC risk. Analyses were repeated for metastases, treated as a secondary outcome. Further secondary analyses were conducted among men who never took a statin after ADT. Race-stratified analyses showed similar findings in black and white men.

Statistical analyses were conducted using STATA version 13.1 (Stata Corp., College Station, TX, USA). Statistical significance was two-sided with $p < 0.05$.

RESULTS

Patient characteristics

Of 302 men, 83 (27%) had elevated cholesterol (≥ 200 mg/dl) (**Table 1**). Men with elevated cholesterol had earlier years of surgery (2007 vs. 2011, $p = 0.004$) and longer follow-up (82.5 vs 60.2 months; $p = 0.035$). Men with normal cholesterol were more likely to have had radiation therapy than patients with elevated cholesterol (74% vs. 58%, $p = 0.005$). There were no significant differences between age, race, PSA or pathological tumor features between total cholesterol groups. Men with elevated cholesterol also had higher LDL, higher HDL, and higher TG (all $p \leq 0.009$). Patients with normal total cholesterol were less likely to start statins after ADT (33% vs. 61%, $p < 0.001$).

Serum lipids and CRPC

During a median follow-up of 61 months (IQR 32-108), 42 patients developed CRPC. Kaplan-Meier plots showed similar time to CRPC between men with normal vs. abnormal total serum cholesterol (**Figure 1**; log-rank, $p = 0.61$), and there was no association between total cholesterol and CRPC risk on multivariable analysis (**Table 2**). Similarly, in secondary analyses, we found no significant associations between LDL or TG levels and CRPC risk (Table 2). However, relative to high HDL (≥ 40 mg/dL), low HDL (< 40 mg/dL) was suggestively associated with increased risk of CRPC (multivariable HR 1.86, 95%CI 0.99-3.48, $p = 0.053$).

Among 178 men who never took a statin while on ADT, total cholesterol and LDL remained unassociated with CRPC risk (**Table 3**). However, on multivariable analysis, higher TG levels as a continuous variable were associated with increased risk of CRPC (HR 1.09, 95%CI 1.02-1.17, $p = 0.010$). Similar to the overall

analysis, there was an increased risk of CRPC among patients with HDL <40 mg/dL who did not start a statin after ADT (multivariable HR 3.64, 95%CI 1.45-9.17, p=0.006).

Serum lipids and metastases

During a median follow-up of 64 months (IQR 33-112), 44 patients developed metastases. There was no association between total cholesterol, LDL or TG levels and risk of developing metastases after ADT (**Table 4**). Relative to patients with high HDL, those with low HDL had increased risk of metastasis (multivariable HR 2.02, 95% CI 1.11-3.70, p=0.021).

Among men who did not start a statin after ADT, there was no association between total cholesterol or LDL and risk of metastasis after ADT (**Table 5**). When treated as a continuous variable, higher TG levels were positively associated with metastasis risk (HR 1.07, 95%CI 1.01-1.13, p=0.027), though no significant association was seen for categorical TG levels. Similar to the overall analysis, low HDL was associated with increased risk of metastasis among men who did not start statins after ADT (multivariable HR 2.64, 95%CI 1.11-6.29, p=0.028).

DISCUSSION

Using data from a retrospective cohort of RP patients subsequently treated with ADT for non-metastatic disease, we found no associations between serum cholesterol or LDL and risk of developing either CRPC or metastases. However, low HDL (<40mg/dl) was significantly associated with increased metastases risk and suggestively associated with increased CRPC risk, with stronger associations among men who remained statin non-users throughout follow-up. High TG was also moderately but significantly associated with increased CRPC and metastasis risk, but only in men who remained statin non-users throughout follow-up. These data suggest that low HDL and high TG may have a role in prostate cancer progression. Further investigations are required to better understand dyslipidemia at ADT as a risk factor for CRPC and/or metastases.

We previously reported that elevated serum TG at RP was associated with increased risk of PSA recurrence, while higher total cholesterol and lower HDL were associated with increased risk of recurrence only among men with dyslipidemia.¹⁶ Others have shown no relationship between serum cholesterol and PSA recurrence after surgery,¹⁷ or even an inverse relationship.^{18, 19} However, potential factors influencing progression to CRPC may differ from those affecting PSA recurrence. Specifically, a key mechanism leading to CRPC development is *de novo* steroidogenesis from intratumoral cholesterol. Indeed, xenograft models, human prostate cancers, and prostate cancer cell lines express various functional *de novo* steroidogenesis-specific enzymes.²⁰ Locke *et al.* showed that *ex vivo* CRPC explants expressed the necessary enzymes to convert the cholesterol precursor, acetic acid to dihydrotestosterone.² Mostaghel *et al.* found that high serum cholesterol increased prostate tumor volume and intratumoral testosterone levels *in vivo*.³ Moreover, in a transgenic mouse model, we found that reducing serum cholesterol lowered tumor androgens and slowed tumor proliferation.⁴ The potential biological significance of cholesterol in CRPC progression provided rationale to test our hypothesis.

Contrary to our hypothesis, we found no association between serum cholesterol, LDL, or TG levels and CRPC or metastasis risk among all men. Nuances between serum vs. tumor cholesterol may underlie our null findings. Specifically, strong data support the importance of altered tumor cholesterol metabolism in prostate cancer progression. For example, an epidemiological study showed that high tumor gene expression of SQLE, the second rate-limiting enzyme in cholesterol biosynthesis, was associated with increased lethal prostate cancer risk.²¹ Recently, our group reported that loss of the p450 sterol hydroxylase, CYP27A1, was common in prostate cancer and that restoration of CYP27A1 or its metabolic end-product 27-hydroxycholesterol reduced intracellular cholesterol accumulation and decreased prostate cancer growth *in vitro* and *in vivo*.²² These data provide evidence that altered cholesterol tumor metabolism may drive lethal prostate cancer and that reducing intratumoral cholesterol could impair prostate cancer growth. However, we found no link between *serum* cholesterol and CRPC risk in the present analysis. It is noteworthy that serum and intratumoral cholesterol are not always correlated. In a prior study, lowering serum cholesterol via ezetimibe had no effect on xenograft growth.²³

Despite lower serum cholesterol, the ezetimibe group had *higher* tumor cholesterol and elevated expression of LDL receptor, potentially a mechanism of resistance to serum cholesterol reduction allowing the tumor to continue to accrue sufficient cholesterol. While simply lowering serum cholesterol may be insufficient to block all prostate cancer growth, these data provide further evidence of the key role of cholesterol in prostate cancer biology. As such, our null findings regarding *serum* cholesterol and CRPC risk do not invalidate the hypothesis that cholesterol is crucial for CRPC, but merely suggest that *serum* cholesterol may not be crucial for CRPC.

Despite our null findings with respect to total cholesterol, our secondary analyses found low HDL was associated with increased metastases risk and suggestively associated with increased CRPC risk, with stronger associations among men who remained non-statin users throughout follow-up. Low HDL is part of the metabolic syndrome (MS), a group of risk factors including high blood pressure, dyslipidemia, high blood sugar and large waist circumference.²⁴ Thus, it is possible that the association between low HDL and CRPC is mediated via other MS components, although in the present study our results were not substantially altered after stratifying models by median BMI or including BMI as an additional covariate in our models. Finally, a recent report by Sekine *et al.* showed that high HDL *promoted* proliferation and migration of androgen-independent prostate cancer cell lines *in vitro*,²⁵ which at first glance appears contradictory to our findings. However, the authors demonstrated that prostate cancer cells uptake HDL more efficiently in low androgen environments via higher expression of ABCA1 receptors, which are normally downregulated by intratumoral androgens.²⁵ More efficient HDL uptake by prostate cancer cells via this mechanism could deplete serum HDL, potentially leading to our observation that low serum HDL is linked with increased CRPC risk. Thus, further studies are required to clarify the complex biology of HDL in the context of CRPC and metastases.

Several study limitations should be considered. First, our sample size was small (n=302), particularly when restricting to statin never-users (n=178), potentially increasing the likelihood of false negative findings. We examined men who received ADT for nonmetastatic disease to create a more homogenous group, and this may limit the generalizability of our results. Moreover, as most men who

developed metastasis also developed CRPC, we cannot tease apart the potential role of cholesterol on each of these outcomes. Future studies would benefit from a larger sample size and longer follow-up in order to ascertain a greater number of events. Secondly, we assessed serum lipid levels in the 2-year period prior to ADT, but lipid levels may change over time and may be influenced by the growing tumor²⁶ and/or by prostate cancer treatment.²⁷ As such, the optimal timing for measuring serum lipids to understand their possible association with CRPC and metastasis is unknown. Moreover, in the context of CRPC, it would be ideal to measure intratumoral cholesterol levels in addition to serum cholesterol as tumor biology may be important for understanding prostate cancer cholesterol metabolism. Again, it would be challenging to track changes over time and thus optimal timing would need to be identified. Finally, though SEARCH is racially diverse, it is largely comprised of earlier-stage patients at lower risk of progression, potentially limiting the generalizability of our findings.

In conclusion, our analyses showed that total serum cholesterol prior to ADT was not associated with CRPC risk in patients with non-metastatic prostate cancer undergoing ADT after RP. However, serum cholesterol may not always accurately reflect intratumor cholesterol, and it may be essential to measure tumor cholesterol metabolism to better understand the intricacies of cholesterol biology. Lastly, our analyses suggested that HDL and TG levels may be associated with risk of metastases and CRPC after ADT, particularly among men who remained non-statin users throughout follow-up, a point that requires further validation.

References:

1. Siegel, R. L., Miller, K. D., Jemal, A.: Cancer statistics, 2019. *CA Cancer J Clin*, 2019
2. Locke, J. A., Guns, E. S., Lubik, A. A. et al.: Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. *Cancer Res*, **68**: 6407, 2008
3. Mostaghel, E. A., Solomon, K. R., Pelton, K. et al.: Impact of circulating cholesterol levels on growth and intratumoral androgen concentration of prostate tumors. *PLoS One*, **7**: e30062, 2012
4. Allott, E. H., Masko, E. M., Freedland, A. R. et al.: Serum cholesterol levels and tumor growth in a PTEN-null transgenic mouse model of prostate cancer. *Prostate Cancer Prostatic Dis*, **21**: 196, 2018
5. Platz, E. A., Clinton, S. K., Giovannucci, E.: Association between plasma cholesterol and prostate cancer in the PSA era. *Int J Cancer*, **123**: 1693, 2008
6. Mondul, A. M., Clipp, S. L., Helzlsouer, K. J. et al.: Association between plasma total cholesterol concentration and incident prostate cancer in the CLUE II cohort. *Cancer Causes Control*, **21**: 61, 2010
7. Bansal, D., Undela, K., D'Cruz, S. et al.: Statin use and risk of prostate cancer: a meta-analysis of observational studies. *PLoS One*, **7**: e46691, 2012
8. Harshman, L. C., Wang, X., Nakabayashi, M. et al.: Statin Use at the Time of Initiation of Androgen Deprivation Therapy and Time to Progression in Patients With Hormone-Sensitive Prostate Cancer. *JAMA Oncol*, **1**: 495, 2015
9. Anderson-Carter, I., Posielski, N., Liou, J. I. et al.: The impact of statins in combination with androgen deprivation therapy in patients with advanced prostate cancer: A large observational study. *Urol Oncol*, **37**: 130, 2019
10. Jeon, J. C., Park, J., Park, S. et al.: Hypercholesterolemia Is Associated with a Shorter Time to Castration-Resistant Prostate Cancer in Patients Who Have Undergone Androgen Deprivation Therapy. *World J Mens Health*, **34**: 28, 2016

- 308 11. Whitney, C. A., Howard, L. E., Freedland, S. J. et al.: Impact of age, comorbidity, and
309 PSA doubling time on long-term competing risks for mortality among men with non-
310 metastatic castration-resistant prostate cancer. *Prostate Cancer Prostatic Dis*, 2018
311
- 312 12. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on
313 Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult
314 Treatment Panel III) final report. *Circulation*, **106**: 3143, 2002
315
- 316 13. Arlen, P. M., Bianco, F., Dahut, W. L. et al.: Prostate Specific Antigen Working Group
317 guidelines on prostate specific antigen doubling time. *J Urol*, **179**: 2181, 2008
318
- 319 14. Scher, H. I., Halabi, S., Tannock, I. et al.: Design and end points of clinical trials for
320 patients with progressive prostate cancer and castrate levels of testosterone:
321 recommendations of the Prostate Cancer Clinical Trials Working Group. *J Clin Oncol*,
322 **26**: 1148, 2008
323
- 324 15. Keto, C. J., Aronson, W. J., Terris, M. K. et al.: Obesity is associated with castration-
325 resistant disease and metastasis in men treated with androgen deprivation therapy after
326 radical prostatectomy: results from the SEARCH database. *BJU Int*, 2011
327
- 328 16. Allott, E. H., Howard, L. E., Cooperberg, M. R. et al.: Serum Lipid Profile and Risk of
329 Prostate Cancer Recurrence: Results from the SEARCH Database. *Cancer Epidemiol*
330 *Biomarkers Prev*, **23**: 2349, 2014
331
- 332 17. Rantaniemi, L., Tammela, T. L. J., Kujala, P. et al.: Blood cholesterol, tumor clinical
333 characteristics and risk of prostate cancer progression after radical prostatectomy. *Scand J*
334 *Urol*: 1, 2018
335
- 336 18. Wettstein, M. S., Saba, K., Umbehr, M. H. et al.: Prognostic Role of Preoperative Serum
337 Lipid Levels in Patients Undergoing Radical Prostatectomy for Clinically Localized
338 Prostate Cancer. *Prostate*, **77**: 549, 2017
339
- 340 19. Ohno, Y., Ohori, M., Nakashima, J. et al.: Association between preoperative serum total
341 cholesterol level and biochemical recurrence in prostate cancer patients who underwent
342 radical prostatectomy. *Mol Clin Oncol*, **4**: 1073, 2016
343
- 344 20. Bennett, N. C., Hooper, J. D., Lambie, D. et al.: Evidence for steroidogenic potential in
345 human prostate cell lines and tissues. *Am J Pathol*, **181**: 1078, 2012
346
- 347 21. Stopsack, K. H., Gerke, T. A., Sinnott, J. A. et al.: Cholesterol Metabolism and Prostate
348 Cancer Lethality. *Cancer Res*, **76**: 4785, 2016

349

350 22. Alfaqih, M. A., Nelson, E. R., Liu, W. et al.: CYP27A1 loss dysregulates cholesterol
351 homeostasis in prostate cancer. *Cancer Res*, 2017
352

353 23. Masko, E. M., Alfaqih, M. A., Solomon, K. R. et al.: Evidence for Feedback Regulation
354 Following Cholesterol Lowering Therapy in a Prostate Cancer Xenograft Model.
355 *Prostate*, **77**: 446, 2017
356

357 24. O'Neill, S., O'Driscoll, L.: Metabolic syndrome: a closer look at the growing epidemic
358 and its associated pathologies. *Obes Rev*, **16**: 1, 2015
359

360 25. Sekine, Y., Demosky, S. J., Stonik, J. A. et al.: High-density lipoprotein induces
361 proliferation and migration of human prostate androgen-independent cancer cells by an
362 ABCA1-dependent mechanism. *Mol Cancer Res*, **8**: 1284, 2010
363

364 26. Kritchevsky, S. B., Wilcosky, T. C., Morris, D. L. et al.: Changes in plasma lipid and
365 lipoprotein cholesterol and weight prior to the diagnosis of cancer. *Cancer Res*, **51**: 3198,
366 1991
367

368 27. Morote, J., Gomez-Caamano, A., Alvarez-Ossorio, J. L. et al.: The metabolic syndrome
369 and its components in patients with prostate cancer on androgen deprivation therapy. *J*
370 *Urol*, **193**: 1963, 2015
371

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Figure legends

Figure 1: Kaplan-Meier plots showing CRPC-free survival according to categories of A) total cholesterol, B) LDL, C) HDL, and D) triglyceride levels

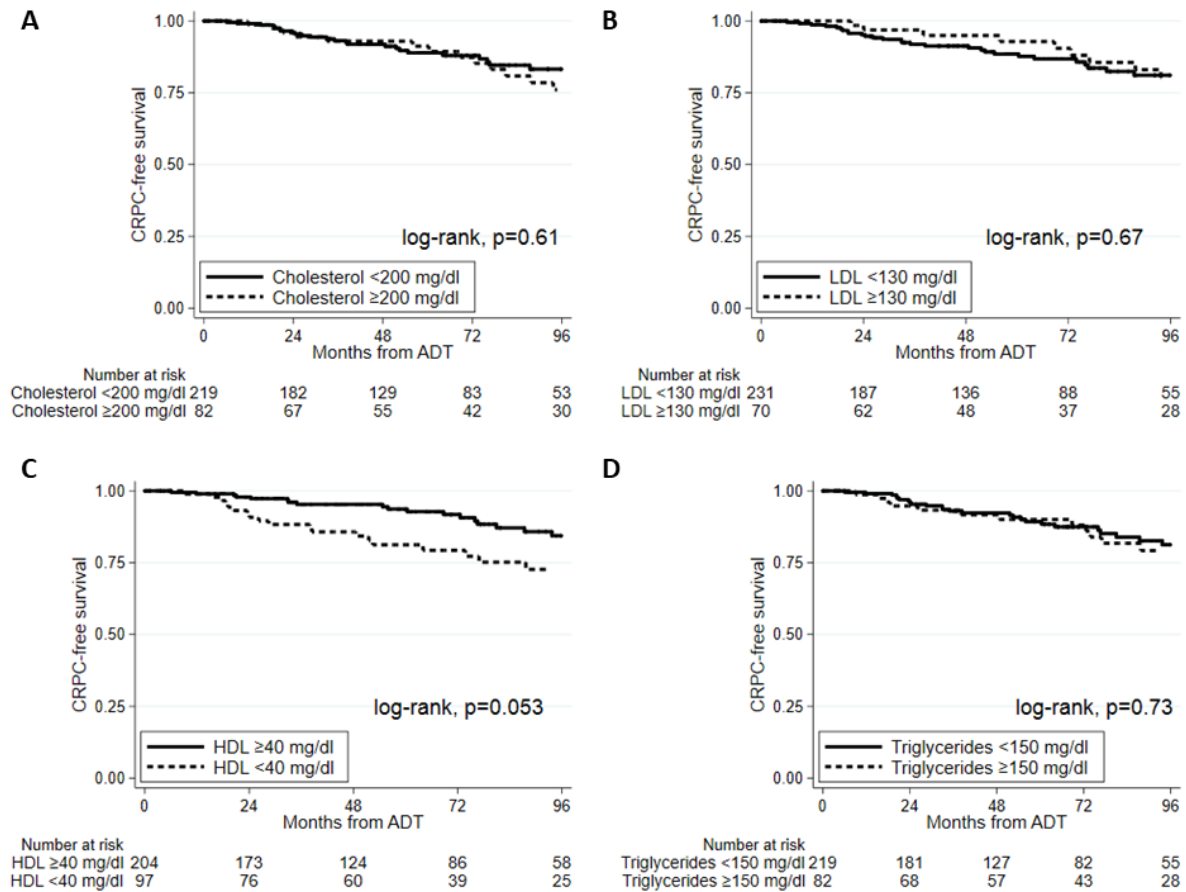


Table 1: Demographic, clinical, and pathological characteristics of patients by serum cholesterol status in the SEARCH database

	Cholesterol < 200 mg/dl (n=219; 73%)	Cholesterol ≥ 200 mg/dl (n=83; 27%)	p-value
Age, median (Q1-Q3)	62 (56-65)	62 (56-66)	0.88 [†]
Race, n (%)			0.37 [§]
Black	89 (41%)	29 (35%)	
Non-black	130 (59%)	54 (65%)	
BMI (kg/m ²), median (Q1-Q3)	27.1 (24.1-30.9)	27.9 (24.7-30.3)	0.36 [†]
Year of RP, median (Q1-Q3)	2007 (202-2012)	2006 (2002-2011)	0.24 [†]
Year of ADT, median (Q1-Q3)	2011 (2006-2014)	2007 (2004-2013)	0.004 [†]

Follow-up, median (Q1-Q3)	60.2 (33.4-106.6)	82.5 (33.9-152.8)	0.035 [†]
PSA at surgery, median (Q1-Q3)*	9.1 (6.1-14.8)	9.8 (7.3-14.8)	0.15 [†]
PSA at ADT, median (Q1-Q3)	0.8 (0.2-3.2)	1.1 (0.3-3.4)	0.18 [†]
PSADT at ADT			0.84 [§]
≥9 months	62 (38%)	26 (31%)	
<9 months	82 (37%)	31 (37%)	
Not calculable	75 (34%)	26 (31%)	
Months from RP to ADT, median (Q1-Q3)	17.7 (4.3-64.9)	10.7 (5.2-47.1)	0.16 [†]
XRT, n (%)	163 (74%)	48 (58%)	0.005 [§]
Statin use, n (%)			<0.001 [§]
Never	150 (67%)	33 (38%)	
Started after ADT	73 (33%)	51 (61%)	
LDL, mean (SD)	98 (80-114)	140 (124-152)	<0.001 [†]
HDL, mean (SD)	44 (35-52)	48 (39-66)	0.006 [†]
Triglycerides, mean (SD)	94 (68-144)	114 (76-192)	0.009 [†]
Pathological grade group, n (%)*			0.096 [§]
1	29 (13%)	6 (7%)	
2-3	118 (54%)	39 (48%)	
4-5	70 (32%)	36 (44%)	
Positive margins, n (%)*	119 (54%)	50 (60%)	0.36 [§]
Extracapsular extension, n (%)*	101 (46%)	44 (53%)	0.30 [§]
Seminal vesicle invasion, n (%)	74 (34%)	27 (33%)	0.84 [§]
Positive lymph nodes, n (%)	36 (16%)	14 (17%)	0.35 [†]

SD=standard deviation; Q1=25th percentile; Q3=75th percentile; PSA=prostate specific antigen;
 BMI=body mass index; RP=radical prostatectomy

*reported among patients with data available

p values calculated by [†] Wilcoxon rank-sum or [§] chi-square test

Table 2: Hazard ratios for the association between serum lipid levels and risk of CRPC after ADT

Total cholesterol	<200 mg/dL	≥200 mg/dL	Continuous[§]
Events/N	27/219	15/83	42/302
Unadjusted	<i>Ref.</i>	1.18 (0.62-2.24), p=0.61	0.99 (0.91-1.09), p=0.35
Adjusted*	<i>Ref.</i>	0.89 (0.47-1.71), p=0.73	0.94 (0.86-1.03), p=0.21
LDL	<130 mg/dL	≥130 mg/dL	Continuous[§]
Events/N	31/232	11/70	42/302
Unadjusted	<i>Ref.</i>	0.86 (0.43-1.72), p=0.67	0.98 (0.89-1.09), p=0.31
Adjusted*	<i>Ref.</i>	0.67 (0.33-1.36), p=0.25	0.94 (0.85-1.04), p=0.23
HDL	≥40 mg/dL	<40 mg/dL	Continuous[§]

Events/N	23/204	19/98	42/302
Unadjusted	<i>Ref.</i>	1.81 (0.98-3.32), p=0.056	0.95 (0.80-1.14), p=0.58
Adjusted*	<i>Ref.</i>	1.86 (0.99-3.48), p=0.053	0.96 (0.81-1.14), p=0.63
Triglycerides	<150 mg/dL	≥150 mg/dL	Continuous[§]
Events/N	29/219	13/83	42/302
Unadjusted	<i>Ref.</i>	1.03 (0.54-1.99), p=0.92	1.00 (0.97-1.04), p=0.89
Adjusted*	<i>Ref.</i>	0.85 (0.44-1.66), p=0.57	1.00 (0.97-1.03), p=0.99

Cells display *hazard ratio (95% confidence interval), p-value*

HDL=high density lipoprotein; LDL=low density lipoprotein

[§]Hazard ratios are for every 10 mg/dL increase

*Hazard ratios are adjusted for PSA at ADT, year of ADT, seminal vesicle invasion, and race

Table 3: Hazard ratios for the association between serum lipid levels and risk of CRPC after ADT in patients who never took statins

Total cholesterol	<200 mg/dL	≥200 mg/dL	Continuous[§]
Events/N	16/146	6/32	22/178
Unadjusted	<i>Ref.</i>	2.20 (0.86-5.65), p=0.10	1.08 (0.94-1.23), p=0.29
Adjusted*	<i>Ref.</i>	1.16 (0.42-3.26), p=0.77	0.97 (0.85-1.10), p=0.62
LDL	<130 mg/dL	≥130 mg/dL	Continuous[§]
Events/N	19/150	3/28	22/178
Unadjusted	<i>Ref.</i>	0.85 (0.25-2.87), p=0.30	1.05 (0.91-1.22), p=0.50
Adjusted*	<i>Ref.</i>	0.51 (0.14-1.86), p=0.30	0.95 (0.81-1.11), p=0.49
HDL	≥40 mg/dL	<40 mg/dL	Continuous[§]
Events/N	11/127	11/51	22/178
Unadjusted	<i>Ref.</i>	2.86 (1.24-6.60), p=0.014	0.91 (0.70-1.17), p=0.45
Adjusted*	<i>Ref.</i>	3.64 (1.45-9.17), p=0.006	0.94 (0.71-1.25), p=0.68
Triglycerides	<150 mg/dL	≥150 mg/dL	Continuous[§]
Events/N	16/142	6/36	22/178
Unadjusted	<i>Ref.</i>	1.64 (0.63-4.24), p=0.31	1.05 (0.99-1.11), p=0.11
Adjusted*	<i>Ref.</i>	1.57 (0.56-4.42), p=0.39	1.09 (1.02-1.17), p=0.010

Cells display *hazard ratio (95% confidence interval), p-value*

HDL=high density lipoprotein; LDL=low density lipoprotein

[§]Hazard ratios are for every 10 mg/dL increase

*Hazard ratios are adjusted for PSA at ADT, year of ADT, seminal vesicle invasion, and race

Table 4: Hazard ratios for the association between serum lipid levels and risk of metastases after ADT

Total cholesterol	<200 mg/dL	≥200 mg/dL	Continuous[§]
Events/N	30/219	14/83	44/302
Unadjusted	<i>Ref.</i>	1.01 (0.53-1.91), p=0.98	0.98 (0.90-1.07), p=0.69
Adjusted*	<i>Ref.</i>	0.85 (0.44-1.63), p=0.62	0.95 (0.87-1.04), p=0.30

LDL	<130 mg/dL	≥130 mg/dL	Continuous[§]
Events/N	33/232	11/70	44/302
Unadjusted	<i>Ref.</i>	0.85 (0.43-1.70), p=0.65	0.97 (0.88-1.07), p=0.99
Adjusted*	<i>Ref.</i>	0.77 (0.38-1.54), p=0.45	0.95 (0.86-1.05), p=0.34
HDL	≥40 mg/dL	<40 mg/dL	Continuous[§]
Events/N	22/204	22/98	44/302
Unadjusted	<i>Ref.</i>	2.17 (1.20-3.92), p=0.010	0.95 (0.79-1.13), p=0.55
Adjusted*	<i>Ref.</i>	2.02 (1.11-3.70), p=0.021	0.96 (0.80-1.15), p=0.65
Triglycerides	<150 mg/dL	≥150 mg/dL	Continuous[§]
Events/N	32/219	12/83	44/302
Unadjusted	<i>Ref.</i>	0.86 (0.44-1.67), p=0.66	1.00 (0.96-1.03), p=0.68
Adjusted*	<i>Ref.</i>	0.71 (0.36-1.40), p=0.33	0.99 (0.95-1.02), p=0.48

Cells display *hazard ratio (95% confidence interval), p-value*

HDL=high density lipoprotein; LDL=low density lipoprotein

[§]Hazard ratios are for every 10 mg/dL increase

*Hazard ratios are adjusted for PSA at ADT, year of ADT, seminal vesicle invasion, and race

Table 5: Hazard ratios for the association between serum lipid levels and risk of metastasis after ADT in patients who never took statins

Total cholesterol	<200 mg/dL	≥200 mg/dL	Continuous[§]
Events/N	19/146	6/32	25/178
Unadjusted	<i>Ref.</i>	1.84 (0.73-4.63), p=0.19	1.06 (0.94-1.20), p=0.36
Adjusted*	<i>Ref.</i>	1.19 (0.45-3.16), p=0.73	1.00 (0.88-1.13), p=0.96
LDL	<130 mg/dL	≥130 mg/dL	Continuous[§]
Events/N	23/150	2/28	25/178
Unadjusted	<i>Ref.</i>	0.46 (0.11-1.96), p=0.29	1.00 (0.87-1.16), p=0.96
Adjusted*	<i>Ref.</i>	0.36 (0.08-1.60), p=0.18	0.94 (0.80-1.09), p=0.38
HDL	≥40 mg/dL	<40 mg/dL	Continuous[§]
Events/N	14/127	11/51	25/178
Unadjusted	<i>Ref.</i>	2.13 (0.97-4.72), p=0.060	0.99 (0.80-1.23), p=0.95
Adjusted*	<i>Ref.</i>	2.64 (1.11-6.29), p=0.028	1.01 (0.79-1.30), p=0.91
Triglycerides	<150 mg/dL	≥150 mg/dL	Continuous[§]
Events/N	20/142	5/36	25/178
Unadjusted	<i>Ref.</i>	1.00 (0.37-2.66), p=0.99	1.03 (0.98-1.09), p=0.27
Adjusted*	<i>Ref.</i>	1.21 (0.42-3.46), p=0.72	1.07 (1.01-1.13), p=0.027

Cells display *hazard ratio (95% confidence interval), p-value*

HDL=high density lipoprotein; LDL=low density lipoprotein

[§]Hazard ratios are for every 10 mg/dL increase

*Hazard ratios are adjusted for PSA at ADT, year of ADT, seminal vesicle invasion, and race